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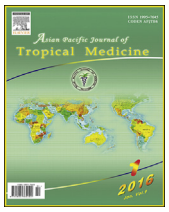

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Bio-ecology of malaria vectors in an endemic area, Southeast of Iran

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ABSTRACT

Objective: To determine some bio-ecological aspects of malaria vectors in Jask County, where is targeted for malaria elimination in the national program.

Methods: Mosquitoes were collected monthly during 2013–2014 using different collection methods. Subsequently, ELISA test was used to detect the human blood index of mosquitoes. The susceptibility status of *Anopheles stephensi* was evaluated against the diagnostic dosages of seven WHO recommended insecticides.

Results: A total of 3650 female and 4736 *Anopheles* larvae were collected including *Anopheles stephensi*, *Anopheles culicifacies* s.l., *Anopheles dthali*, *Anopheles fluviatilis* s.l., *Anopheles moghulensis* and *Anopheles turkhodi* species. *Anopheles stephensi* was the dominant collected species on human baits and indoors with high rate of unfed and gravid specimens in internal and external window traps. Human blood index was calculated as 14.3% for this species. It was also found to be resistant to DDT and Dieldrin.

Conclusions: The collected species had a wide range of habitats, and resting behaviors. With regarding to the presence of most important malaria vectors in Jask, control of the disease may be so complicated; as based on the weather condition it can be transmitted during the whole year, expect for cold months. With this strong potential of transmission, existing population movements in the area may lead to imported cases of malaria and local outbreak(s). So, more specific studies on malaria vectors in high risk areas of Jask County are recommended.

1. Introduction

Malaria is the main vector-borne infectious diseases in the world with more about 584000 deaths during 2013, 90% of them occur in African countries, and in children aged less than 5 years [1]. The disease vector is *Anopheles* mosquito and about 70 species of this insect have capacity to transmit malaria [2]. To combat the malaria transmission and vectors it is necessary to understand the behavior of the vector(s) species. Spatial distribution of mosquitoes highly depends on the environmental and climatologically factors, so each species has its own range in a given area. Knowledge about the fauna and bionomics of malaria vectors in a given area is a very important issue in the assessment of malaria risk and planning vector control and prevention. Moreover seasonal changes in vector population are important to determine their population size and peak(s) of activity and implementing control measures [3]. Also when the study area has more than one climate, the survey should cover different climates to better understanding the malaria epidemiology and transmission dynamics [4].

The main malaria vector control tools are indoors residual spraying (IRS) and long lasting insecticide impregnated bed-

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nets. Correct using these tools highly depends on mosquitoes biting and resting habitats as well as their susceptibility to the applied insecticides. Extensive and long term using of chemicals in malaria vector control programs led to change in vector behavior [5–7].

Based on the WHO classification, Iran is now in the elimination stage for malaria control with 1 373 confirmed cases in 2013. About 746 000 residents are living in malaria active foci of the country [1]. Two *Plasmodium* species are reported from malaria patients in Iran: *Plasmodium vivax* (82%) and *Plasmodium falciparum* (18%). Seven *Anopheles* species are introduced as main and secondary vectors in the country: *Anopheles stephensi* (*An. stephensi*), *Anopheles culicifacies* s.l. (*An. culicifacies* s.l.), *Anopheles fluviatilis* s.l. (*An. fluviatilis* s.l.), *Anopheles dthali* (*An. dthali*), *Anopheles superpictus* s.l., *Anopheles maculipennis* complex and *Anopheles sacharovi*. The first five species are responsible for malaria transmission in southern parts, where active foci of the disease are available.

Nowadays, malaria transmission and indigenous cases occur mainly in three Provinces including Sistan va Baluchestan, Hormozgan and Kerman. Malaria has a long history in Hormozgan Province and is a major public health problem in some areas. Jask County, a less developed area in the eastern part of the province, contains active foci of malaria, where transmission occurs. On the other hand, it has been the most important malarious area in Hormozgan province during current years [8]. With due attention to the lake of formal information about malaria vectors in the study area, their monthly activity and abundance, human blood index, mosquito vector behavior and susceptibility status to insecticides, this study was aimed to determine the bio-ecology of malaria vectors in Jask County.

2. Materials and methods

2.1. Study area

Jask County with a population of 56 788 is located in eastern part of Hormozgan Province, southern Iran. The county is distributed between 57° 10′–59° 16′ E and 25° 23′–26° 13′ N. Jask is bordered with Sistan va Baluchestan Province (East and Northeast), Bashagard County (North), Sirik County (Northwest and West) and Oman Sea (South). The weather in Jask is dry and warm with hot summers and temperate winters. In 2013 the average of minimum and maximum monthly temperature were recorded as 13 °C and 39.6 °C in January and May, respectively. The county can be divided into two parts: more than 90% of the area is located in plain/coastal area with less than 400 m, and 10% has an altitude of more than 400 m and covered by mountains. Generally, the most villages of county are located in lowland area (100 m above sea level) (Figure 1). Weather in southern part of the county is warmer than northern mountainous areas. A total precipitation of 135.3 mm was recorded in 2013 as well. The altitude of Jask County is −23 to 1 900 m above the sea level.

2.2. Entomological studies

This part of the study was conducted monthly for 12 months from June 2013 till June 2014 in four selected villages. The villages were located at the following coordinates and altitudes: 58.172 8 °E, 25.792 9 °N, 45 m; 58.139 8 °E, 25.896 6 °N, 83 m; 58.087 0 °E, 25.950 9 °N, 85 m and 58.336 0 °E, 25.954 8 °N, 115 m. The last village was located in mountainous area but other in plains.

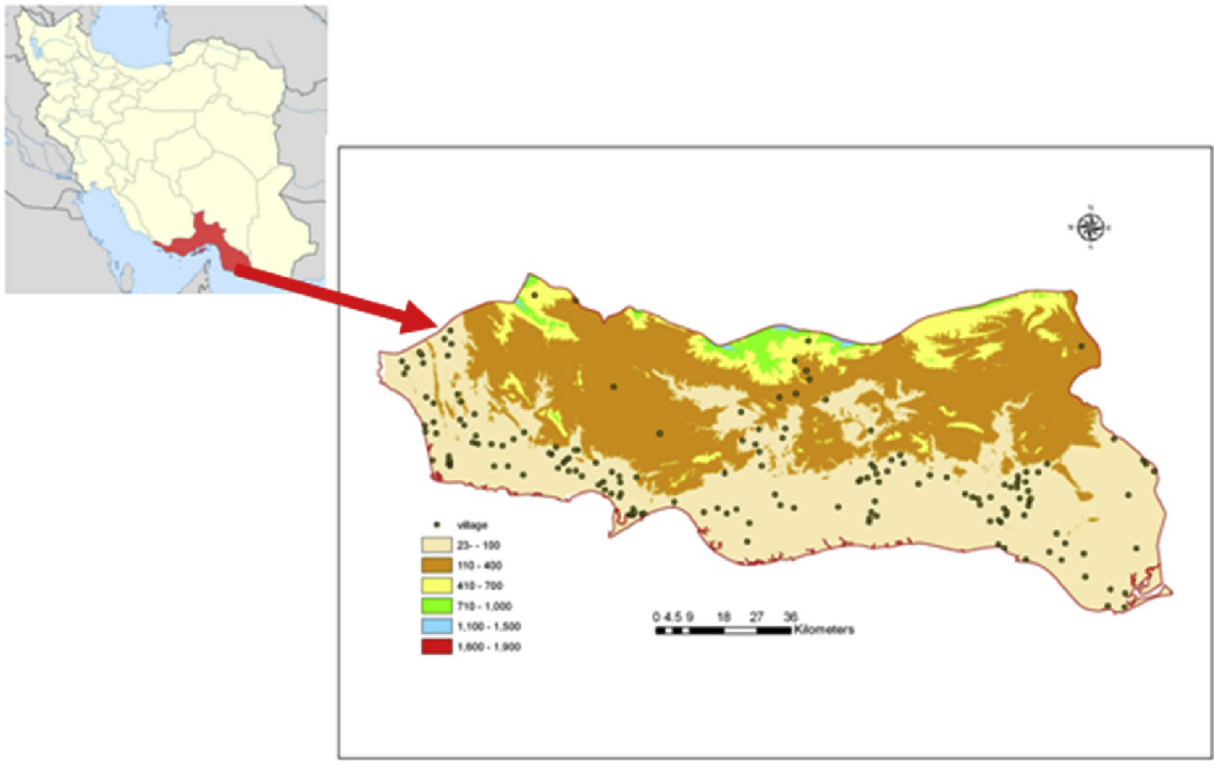

Figure 1. Distribution of villages (dots) in Jask County in Hormozgan Province, Southern Iran. The majority of villages are located in plain/costal area.

According to guideline of World Health Organization [9] sampling sites in each village was selected based on fixed and temporary shelters and mosquito collection was performed monthly. The methods of collection were spray sheet collection, hand catch, artificial pit shelter, window traps, human and animal bait landing collection for adults collection as well as dipping for larvae. Coordinates of collection sites were recorded by GPS device. The larval collection was carried out in from different breeding places, mainly in riverin and river beds. Finally, all samples were identified according to morphological keys [10,11].

All collected Anophelines were identified to species and blood meals of freshly fed females smeared on Whatman filter paper which was allowed to dry. These were packed inside plastic bags and kept at −20 °C until used.

2.3. Blood meal identification

The tests were performed as described by Edrissian *et al*. [12] as follows: the dried spots of blood on the papers were cut to make small discs, 2–3 mm in diameter. Each disc was put in a well of a Micro ELISA plate (NUNC Co. Denmark). The dried blood on the filter paper was eluted with 50 μL of distilled water in each well for 2 h at room temperature. Then 50 μL of coating buffer (carbonate bicarbonate, pH 9.6) was added to each well. The filter papers were stirred inside the wells and removed and then the plates were left overnight at +4 °C inside a humid box. The plates were washed with phosphate buffered saline-Tween 20 (pH 7.2) three times. Then amounts of 50 μL diluted goat anti-human IgG conjugated to alkaline phosphatase were added onto each well, incubated at 37 °C for 2 h and washed as before. Then 100 μL of substrate solution (1 mg/mL P-nitrophenyl phosphate, Sigma, in 10% diethanolamine buffer pH 9.8 containing 0.5 μM $MgCl_2$ and 0.02% NaN_3) was added to each well and left in a dark chamber at room temperature for 30 min. As controls, two wells were left blood free (blank) and two wells were treated with human blood (positive control). The results were assessed by examination with the naked eyes and also absorbance was measured with an ELISA reader at 405 nm about 30 min after the addition of the substrate solution. The test well was considered positive if it gave a visible yellow color.

2.4. Biological forms of An. stephensi

Gravid mosquitoes collected in baited landing catch were transported alive to separate tubes including wet filter paper and allowed to lay eggs. The egg characteristics of 10–12 eggs from each female, including their length, breadth, and number of ridges on the egg float were measured under compound microscope. Measurements were carried out using an ocular micrometer in a stereoscopic microscope at 15 × power, and the number of ridges along one side of the egg float was counted at the same time. All the procedures were followed as described previously [13,14]. Ridge number of 10–15 is considered as Mysorensis, 15–17 as Intermediate and 17–22 as Type forms [13,14].

2.5. Susceptibility tests to insecticides

In addition to measuring the larvae density during routine entomological survey, live larval collection was carried out to provide a colony of adults mosquitoes for bioassay test against selected insecticides. The collected larvae from field were transferred to an insectary under standard condition at Light: Dark = 14:10 h; Temperature: (26 ± 2) °C; Relative humidity: 70%–80%. Subsequently the susceptibility tests were conducted using WHO recommended method [15]. Three to five days old sugar fed females (F1 generation) were used against the diagnostic dose of insecticides following the standard WHO resistance tube assay. Bioassays were performed at a temperature ranging from 26 °C to 28 °C with 70% relative humidity for 60 min. For each insecticide, a total of 100 female mosquitoes were tested in insecticide susceptibility bioassays and 50 for control, with 25 mosquitoes per tube.

The following insecticides were used for the tests: DDT 4%, Dieldrin 0.5%, Malathion 5%, Fenitrothion 1%, Bendiocarb 0.1%, Propoxur 0.1% and Deltamethrin 0.05%. Abbott's formula was used to correct the results with control mortality rates between 5% and 20% [16]. All results with more than 20% mortality in control were discarded.

3. Results

A total of 3 650 female anopheline mosquitoes were captured using different collection methods including spray sheet collection (14%), hand catch (29%), window trap (6%), pit shelter (10%), animal bait landing collection (27%) and human bait landing collection (14%). During study period totally, six *Anopheles* species were found in the study area, *i.e. An. stephensi*, *An. culicifacies* s.l., *An. dthali*, *An. fluviatilis* s.l., *Anopheles turkhodi (An. turkhodi) and Anopheles moghulensis* (*An. moghulensis*).

3.1. Spray sheet collection

An. stephensi was the dominant species (43.9%) collected from indoor places which followed by *An. culicifacies* s.l. (20.1%) and *An. dthali* (18.1%). Two mosquito population peaks were occurred in March–April and September–November (Figure 2), while the highest density of each species was observed in April.

3.2. Hand catch collection

Overall 1 044 female *Anopheles* were collected using this method. The most density of this species was found to be in

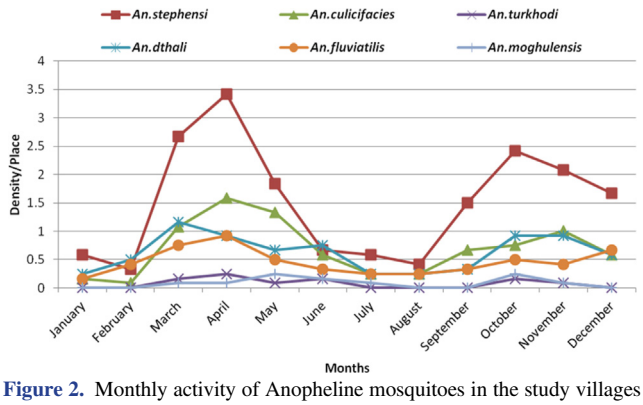

Figure 2. Monthly activity of Anopheline mosquitoes in the study villages collected by spray sheet collection method, Jask County, Southeastern Iran, 2013–2014.

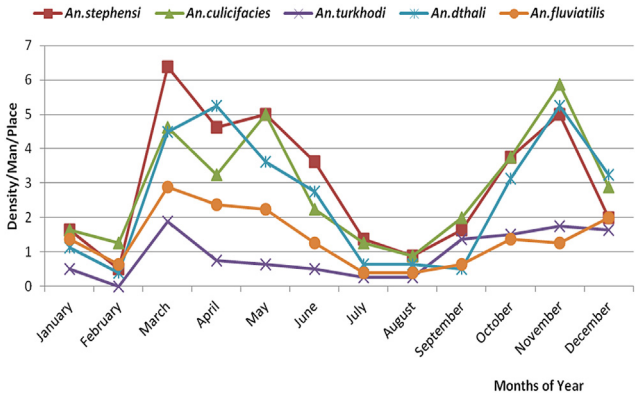

Figure 3. Monthly activity of Anopheline mosquitoes collected by hand catch method in the study area, Jask County, Southeastern Iran, 2013–2014.

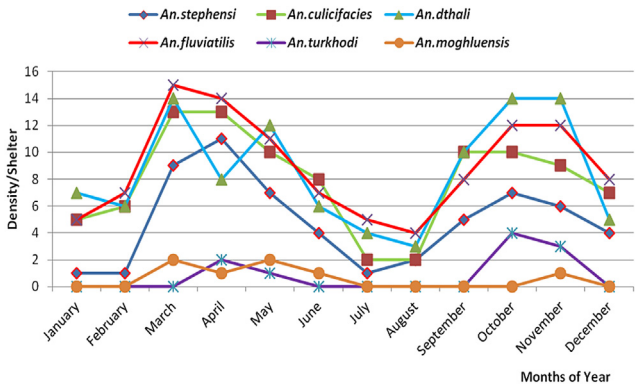

Figure 4. Monthly activity of Anopheline mosquitoes collected in artificial pit shelters of the study area, Jask County, Southeastern Iran, 2013–2014.

March. Regarding to dominancy of collected species by this method *An. stephensi* was the most abundant species with ratio of 27.9% followed by *An. culicifacies* s.l. and *An. dthali*. Two peaks of activity were found in March and November (Figure 3).

3.3. Outdoor collection

A total of 364 female *Anopheles* was collected from outdoor shelters by hand catch method. *An. fluviatilis* s.l. was found to be the dominant species (29.7%), with the highest density in March. Other species were *An. dthali* (28.3%), *An. culicifacies* s.l. (26.1%) and *An. stephensi* (15.9%). The most propensity to rest in outdoor shelters was observed in March and October–November (Figure 4).

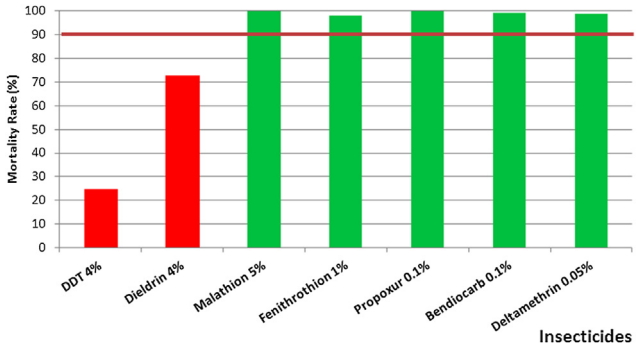

Figure 5. Result of susceptibility status of *An. stephensi* to the diagnostic dose of insecticides in 1 h contact period, Jask County, Southeastern Iran, 2013–2014.

3.4. Landing catch collection

This method was conducted using animal (donkey) and human baits in the study area. Overall four *Anopheles* species were captured: *An. stephensi*, *An. culicifacies* s.l., *An. dthali* and *An. fluviatilis* s.l. Most specimens were collected in the first trimester of the night (18:00–22:00) on animal (6.2%) and human (59.7%) baits. *An. stephensi* was the dominant species in this method followed by *An. culicifacies* s.l. More than 41% of mosquitoes were collected in Fall, followed by Spring, Summer and Autumn, respectively.

3.5. Window traps

Totally 80 and 130 *Anopheles* mosquitoes were collected from internal and external window traps, respectively (Table 1). Four vector species, *i.e. An. stephensi*, *An. culicifacies* s.l., *An. dthali* and *An. fluviatilis* s.l. were collected.

3.6. Human blood index

A total of 288 blood fed *Anopheles* including 56 *An. stephensi*, 79 *An. culicifacies* s.l., 75 *An. dthali* and 78 *An. fluviatilis* s.l. were subjected for identification of the type of fed blood using precipitating tests. Results showed HBI of 14.3%, 13.9%, 14.8% and 17.9% for *An. stephensi*, *An. culicifacies* s.l., *An. dthali* and *An. fluviatilis* s.l., respectively.

Table 1
Anopheles species collected in the window traps, Jask County, Southeastern Iran, 2013–2014 (*n*%).

Trap position	Species	*n*	Abdominal condition			
			Unfed	Blood-fed	Semi-gravid	Gravid
Entrance trap	*An. stephensi*	19	6 (31.6)	6 (31.6)	2 (10.5)	5 (26.3)
	An. culicifacies s.l.	27	8 (29.6)	12 (44.5)	2 (7.4)	5 (18.5)
	An. dthali	21	8 (38.1)	6 (28.6)	4 (19.0)	3 (14.3)
	An. fluviatilis s.l.	13	4 (30.7)	5 (38.5)	2 (15.4)	2 (15.4)
Exit traps	*An. stephensi*	63	4 (6.3)	10 (15.9)	11 (17.5)	38 (60.3)
	An. culicifacies s.l.	37	6 (16.2)	5 (13.5)	5 (13.5)	21 (56.8)
	An. dthali	19	4 (21.1)	5 (26.3)	0 (0.0)	10 (52.6)
	An. fluviatilis s.l.	11	2 (18.2)	4 (36.4)	2 (18.2)	3 (27.2)

Table 2
Anopheles larvae collected in the study area, Jask County, Southeastern Iran, 2013–2014.

Species	January	February	March	April	May	June	July	August	September	October	November	December	Total	Percent
An. stephensi	55	34	263	233	175	96	51	95	151	273	250	120	1 796	37.9
An. culicifacies s.l.	34	27	100	150	142	41	21	39	57	101	168	68	948	20.0
An. dthali	17	18	100	158	155	49	11	12	29	71	100	50	770	16.3
An. fluviatilis s.l.	11	11	86	70	48	24	15	10	16	59	74	49	473	10.0
An. turkhodi	13	8	32	37	25	21	9	2	16	37	61	18	279	5.9
An. moghulensis	0	15	77	139	107	21	4	0	7	55	45	0	470	9.9
Total	130	113	658	787	652	252	111	158	276	596	698	305	4 736	100.0

3.7. Biological forms

A total of 778 eggs randomly obtained from 89 individuals of *An. stephensi*, collected from four studied villages, were examined for detection of the biological forms. The results showed an egg ridge average of 12.43 ± 1.72. Based on Subbarao's categorizing described in methodology of this paper, this average indicated that the geographical population *An. stephensi* in the study area was Mysorensis.

3.8. Susceptibility tests

A total of 1 451 females of *An. stephensi* were evaluated for their susceptibility status to the diagnostic dosages of 7 insecticides. Results showed resistance to DDT 4% and Dieldrin 0.5% with mortality rates of 24.8% and 72.8%, respectively. Other insecticides caused mortality rates more than 98% and therefore this species was considered to be susceptible to malathion, fenitrothion, propoxur, bendiocarb and deltamethrin (Figure 5).

3.9. Larval collection

A total of 4 736 3rd and 4th instars *Anopheles* larvae comprising 6 species were collected from the study area. *An. stephensi* (37.9%) and *An. turkhodi* (5.9%) were found to have the maximum and minimum specimens among collected sample relatively (Table 2). Generally the most density of larvae was found in March–May and September–November indicating there are two peaks of activity for anopheline mosquitoes in the study area.

4. Discussion

The seasonal activities of *Anopheles* mosquito was observed for more than 9 months and presence of three main vectors in the Jask area indicating that the area is potentially in high risk of malaria transmission. Beside of electrical power supply and using air-conditions and also distributing mosquito nets among residents of Jask, the results of current study show the human blood index is relatively high among the vectors. In addition, we found that the main vector, *An. staphensi* is still resistance to DDT and dieldrin but sensitive to malathion, fenitrothion, propoxur, bendiocarb and deltamethrin. The last two insecticides are using in the national malaria vector control program and therefore this findings show they can be used yet, but annual susceptibility tests are recommended before any vector control program in future.

This area with specific geographical, topographical and economic situation has a great potential for the development of mosquitoes and malaria transmission. As most of villages are located in a buffer of 500 m from the permanent/seasonal rivers, close contact of human-*Anopheles* is inevitable. This buffer is confirmed as the flight range of most Anopheline species [17]. We found most of breeding places are located in reveries and riverbeds. After every rainfall temporary water bodies around the villages provide suitable breeding places for mosquitoes. Most of agricultural activities are focused on the riversides, as we found, providing suitable resting places for exophilic species like *An. fluviatilis* s.l. and *An. dthali*. Human dwellings were also provided resting/blood feeding places for endophilic/endophagic *Anopheles* species. During July–August the average of maximum monthly temperature increases to 34–36 °C, while the relative humidity drops to about 30%. It seems this environmental condition decreasing the longevity of Anopheline mosquitoes, so that their population decreased. In some mountainous villages of our study area, some inhabitants used their unprotected sheds for sleeping at night during warm months. Although the population of mosquitoes has decreased in these months, some exophilic/exophagic species obtain good opportunity to take human blood, and therefore they may have more important role in malaria transmission in warm months. *An. stephensi*, a mostly endophilic and endophagic species, and *An. culicifacies* s.l., a species with both endophagy and exophay tendencies [18] can transmit malaria during the year. However, all four above mentioned *Anopheles* species were collected more or less during all months of the year in Jask.

Another main reason for considering this County as a high-risk area for malaria transmission is traffic of foreign immigrants-mostly from Pakistan-without any border quarantines formalities [7], so that the rate of imported comparing to indigenous cases was increasing during past years. In this survey we collected 4 out of 7 malaria vectors of Iran, *i.e. An. stephensi*, *An. culicifacies* s.l., *An. dthali* and *An. fluviatilis* s.l. These species have different resting and blood feeding behaviors [12,19,20]. Therefore, presence of these vectors cause the malaria transmission became more complex process in this area. Regarding the activities of *Anopheles* during whole year in the study area, malaria transmission may occur during whole year, except in January–February, because reduction in density, mosquito-human contact and blood-feeding rate. Although there are rare cases of the disease during these months, most of them seem to be due to relapse, incomplete treatment or in some cases drug resistance in *Plasmodium falciparum*.

Generally, total number of unfed and fresh-fed female mosquitoes found in entrance trap was relatively two third more than gravid and semi-gravid mosquitoes. In contrary, two third of caught females in excite traps had semi gravid and gravid

abdominal condition. *An. culicifacies* was dominant species among collected mosquitoes in entrance trap while half of *Anopheles* mosquitoes found in exit traps, were *An. stephensi.* In our study more than 80% of collected *An. stephensi* was from entrance window traps which mostly were semi-gravid and gravid. This indicated that high endophilicity of this species in the study area. However, in recent years some indications of behavioral resistance observed in the areas where long lasting impregnated bednets are distributed. So that a sizable portion of samples collected from outdoor resting places such as pit shelter was due change in resting behavior of this species [6]. We also found 15% of the samples from outdoor shelters were *An. stephensi*. This may be partially due to the above mentioned issue. Human blood index (HBI) for this species in the study area was 17.9%, which is relatively high. Although, the study villages were supplied with electrical power and the most of residents' houses equipped with air-conditioners, still we found the high rate of HBI. It seems to be due to low population of livestock in the area as well as living-resting behavior of people. Previous studies in other parts of Iran showed this index as 0.5%–19.8% [19,21–23]. Just Mysorensis form of *An. stephensi* was found to be distributed in the study area. This form was also reported from Sistan va Baluchestan Province [24]. Mysorensis biological form seems to have higher capacity for malaria transmission in southern Iran [25].

We found that, the second species in the case of density was *An. culicifacies* s.l. This species has been reported as malaria vector in the epidemics of Zabol County, eastern Iran [17] and has the main role in most parts of Baluchestan area [26,27]. Other studies showed a HBI of 1.18%–20.00% and sporozoite rate of 1.0%–4.7% for this *Anopheles* [17,19,26,27]. We found the HBI rate equal to 13.9% in Jask. Our study area is bordered from the east by Baluchestan area.

Two other species collected in our study, *An. fluviatilis* s.l. and *An. dthali*, are introduced as main and secondary vectors in southern Iran, respectively. Sporozoite and HBI rates of 1.0%–20.8% and 1%–25% are respectively reported for *An. dthali* [19,28–30]. We found HBI rate of 14.7% for this species in this study. *An. fluviatilis* s.l. is an exophilic species with a report of HBI equal to 62.3% [31]. In our study 17.9% of examined sample due to this species were fed on human blood.

Salehi *et al*. [32] found malaria had a positive correlation with temperature and relative humidity, and a negative correlation with rainfall in Sistan va Baluchestan. This correlation can be studied in Jask County because they are in the same climate. Rain storms are happening in both areas and most breeding places are located in reveries/riverbeds. This situation has a negative effect on the density of larvae soon after rainfall. In some areas with permanent breeding places, temperature and relative humidity have more important roles on the longevity of vectors. The same situation is reported in some similar areas [33]. Maximum temperature, average of relative humidity and number of malaria cases in past were found to be the most important indices in malaria prediction [34].

We observed two peaks in monthly activity of Anopheline mosquitoes. This pattern is the same as observations in lowlands/coastal areas of southern Iran. Regarding the altitudinal zoning in Jask County, it can be seen that more than 95% of the villages are located in lowlands with a maximum altitude of 400 m above the sea level. So, it is expected to have two peaks of activity for Anopheline in Jask County. This finding can be used in exact timing of spraying for malaria vector control.

We found the most of malaria vectors were seeking a human host for blood feeding in the 1st trimester of the night. In this period of time, inhabitants were usually awake and a part of them perform their daily tasks out of bed-nets. This behavior increases the man-vector contact and should be noted in educational programs to decrease the risk of malaria transmission.

According to the results of our bioassay tests against different insecticides, *An. stephensi* was resistant to DDT 4% and dieldrin 0.5%. Resistance of this species to DDT, dieldrin and malathion was reported in some other studies in southern Iran [33,35–38]. National malaria program uses Deltamethrin for IRS, while Olyset® LLNs (impregnated with Permethrin) was widely distributed in the malaria foci of Iran. Although some indication of pyrethroid resistance was found in *An. stephensi* in Iran. Our results showed this species was still susceptible to pyrethroid in the study area. Although resistance to malathion was reported after its' application in IRS interventions in the country, it seems that the resistant population of *An. stephensi* is disappeared from some parts of the country [39]. However, our results showed full susceptibility of *An. stephensi* to malathion in Jask County as well.

In conclusion, this is the first formal study on the ecology of malaria vectors in Jask County, which can fill the gap of information in this important area. Due to high potential of malaria transmission in the County, some cases of local transmission and focal epidemics has been occurred during past years. Based on the finding of this study, the correct time for IRS in the areas under vector control measure can be determined. This will suppress the mosquito population timely and reduce the potential of malaria transmission. Also, based on activity of exophilic vectors, using long lasting insecticide treated nets as well as larviciding should be considered accordingly.

Conflict of interest statement

Authors declare no conflict of interest.

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